ABSTRACT

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Title of Diploma Thesis: Application of supercritical fluid chromatography in analysis of natural compounds

The goal of this diploma thesis is to verify the suitability of UHPSFC method with PDA and MS detection for analysis of natural products. For the analyzes, two representatives of natural compounds – the alkaloids of the family *Amaryllidaceae* with effect in the treatment of Alzheimer´s disease and phenolic compounds with antioxidant activity – flavonoid rutin, quercetin and their metabolites were chosen.

Separations were carried out on the UHPSFC system Acquity UPC^2 with PDA and MS detector. The basic screening was performed on 10 stationary phases: BEH 2-EP, HSS SB C18, BEH, Torus DIOL, Torus 2-PIC, Torus DEA, CSH PFP, Torus 1-AA, Cortecs HILIC and BEH Amide. Four mobile phases were tested: 1. CO_2 + MeOH, 2. CO_2 + MeOH + 0.1 % NH_4OH, 3. CO_2 + MeOH + 10 mM ammonium formate, and 4. CO_2 + MeOH + 10 mM ammonium formate + 2 % H_2O. Separation conditions were as follows: flow rate 1.5 ml/min, temperature 40 °C, pressure 140 bar, gradient elution from 2 % to 40 % within 4 minutes, with two isocratic steps at the beginning and at the end of the gradient. Detailed optimization of the chromatographic conditions involved changing the composition of the mobile phases, changing the gradient elution, and the pressure and temperature settings.

The UHPSFC method was developed for both groups of natural compounds and the conditions were optimized to achieve the best selectivity. In the alkaloid group, effective separation of isomers was a critical issue. In the case of phenolic compounds, the problem of elution and separation within one analysis had to be solved due to their different physicochemical properties. For the *Amaryllidaceae* family alkaloids, analysis on a Torus DEA column with CO_2 + MeOH/ACN (1:1) + 0.1 % NH_4OH mobile phase was the most suitable, and allowed separation of all analytes. The separation of phenolic compounds was achieved with Torus DIOL column with a mobile phase of CO_2 + MeOH + 0.05 % NH_4OH + 2 % H_2O. However the elution of quercetin and its glycoside rutin remained subject of future optimization. Targeted analysis of both

groups of natural compounds can be used to explain their metabolism and health effects on the human body.

Keywords: alkaloids of the *Amaryllidaceae* family; phenolic compounds; rutin; quercetin; UHPSFC-PDA/MS; method development; optimization of chromatographic conditions